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# EFFECT OF CERTAIN DEFICIENCIES ON NITROGEN METABOLISM OF PLANTS

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**DISSERTATION**

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE GRADUATE SCHOOL OF THE OHIO STATE UNIVERSITY

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BY

**ROBIN CHARLES BURRELL, B.S., M.A.**

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# PREFACE

Soybean and pumpkin seedlings were grown in trays of washed quartz sand supplied with nutrient solutions of known compositions. The control solution had a composition similar to dilute Knop's solution. The deficient solutions lacked Mg, K, and Ca respectively but had the same physiological balance of all the other essential elements as the control solution. Five days after the seedlings appeared above ground, the cotyledons were removed and analyzed. At the end of 35 days above ground the leaves and stems of the control plants and of each of the deficient series of plants were analyzed separately. Alcoholic (80%) preservation and extraction were used and insoluble residue, solids of extract, insoluble, soluble nitrate, nitrite, ammonia, amide, and amino acid nitrogen, reducing sugars, sucrose, and starch were determined quantitatively. The analyses of the control and of the deficient plants are compared.

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# EFFECT OF CERTAIN DEFICIENCIES ON NITROGEN METABOLISM OF PLANTS

R. C. BURRELL

## Introduction

Very little is known concerning protein synthesis in plants. The intermediate steps involved in the building of these compounds from the simple plant nutrients have never been satisfactorily worked out.

There is evidence that some of the mineral elements recognized as essential for plant growth play important rôles in certain stages of the protein synthesis. For example, SCRUTI (5) has attributed to phosphorus an important rôle in higher plants in the formation of the amino acids; and recently, KRAYBILL (4) has obtained some interesting results in an extensive study of the relation of phosphorus to the nitrogen metabolism of the tomato. Limiting the available quantities of such elements should throw the ordinary leaf machinery out of gear, permitting certain intermediate products of protein synthesis to accumulate, while other substances would cease to be formed, or at least would be produced in only abnormally small amounts. If lack of a certain mineral element, as calcium, would retard or stop the protein synthesis at a different stage from that caused by the lack of some other essential element, as potassium, then intermediate products of varying degrees of complexity might be determined; also some definite functions of these essential elements would be established.

In this investigation the effects of magnesium, potassium, and calcium deficiency on the composition of certain plants were studied. The analyses were compared with analyses of control plants grown side by side with the deficient plants, but supplied with all the essential nutrient elements.

## Experimental procedure

Soy beans (Peking) and pumpkins (Connecticut Field) were the plants selected for the experiments, because they were found to make a rather rapid growth and to thrive moderately well in the green-

house. Navy beans were tried at first, but when grown in sand cultures they were found to be very susceptible to a bacterial wilt, apparently carried by the seed. The plants were grown in trays filled with washed quartz sand. The bottoms of the trays were brushed over on the inside with melted paraffin, the moist sand put in, and the seeds planted in rows three inches apart and one-half inch apart in the rows. Subsequent watering was carried out with the diluted nutrient solutions described later. As required, the same quantities were added to each tray in the same way.

To take into account the effect of greenhouse conditions and other factors that could not be definitely controlled, the trays were alternated with trays of control plants on the greenhouse bench. The development and composition of the deficient plants and of the control plants were then compared. Since all external conditions were approximately the same in the cases of the plants compared, except that one element was deficient, the differences observed were assumed to be due to this deficiency.

Five days after the seedlings appeared above ground, the cotyledons were removed in order that the effects of the deficiency might appear sooner. Thirty days after this (35 days above ground) the plants were sampled. Definite numbers of average plants were removed from the deficient and control trays and stripped of their leaves (petioles removed). These leaf samples were then weighed and preserved in 80 per cent alcohol, with the addition of calcium carbonate, as later described. The stems, from the roots to the point of attachment of the cotyledons, were preserved similarly as separate samples.

The nutrient solutions used were similar to those employed by Dickson (2), except that they were recalculated to an absolute deficiency of the element in question, instead of merely reducing it to one-tenth of that in the normal solution used. This is essentially Knop's solution, diluted one to ten, with corresponding deficient solutions containing exactly the same physiological balance of all the other essential elements except the one that is lacking. The dilution is necessary to take care of the accumulation of salts in sand cultures watered with nutrient solutions. Chemically pure reagents were used. Calcium nitrate and magnesium sulphate solutions were pre-

pared by making approximate solutions from the hydrates, determining the concentration of calcium and magnesium in each by analysis, and then making appropriate dilutions. A block tin condenser was used in the preparation of the distilled water, and solutions were stored in seasoned alkali treated flint glass bottles. Stock solutions of the various salts were prepared which served during the whole experiment. The hydrogen-ion concentrations of the diluted solutions, as actually applied to the plants, varied between 4.5 and 5.0 $P_H$. The osmotic pressure of each solution was approximately 0.0151; that of the calcium deficient solution was very slightly

TABLE I

CONCENTRATIONS OF SALTS IN NUTRIENT SOLUTIONS (GM. PER LITER)

| | NORMAL | Mg DEFICIENT | K DEFICIENT | Ca DEFICIENT |
|---|---|---|---|---|
| Calcium nitrate | 0.533 | 0.533 | 0.533 | ..... |
| Potassium nitrate | 0.133 | 0.133 | ..... | 0.133 |
| Potassium phosphate | 0.133 | 0.133 | ..... | 0.133 |
| Magnesium sulphate | 0.133 | ..... | 0.133 | 0.133 |
| Sodium chloride | 0.067 | 0.070 | 0.063 | ..... |
| Sodium nitrate | ..... | ..... | 0.111 | 0.552 |
| Sodium sulphate | ..... | 0.1576 | ..... | ..... |
| Sodium phosphate | ..... | ..... | 0.117 | ..... |

higher. The concentrations of the diluted solutions are given in table I. Two and five-tenths cc. M–100 ferric chloride solution was added to each liter of nutrient solution just before applying to the plants.

When the plants had grown 35 days above ground, they were sampled as described. Sufficient data were recorded so that the composition of the plants could be expressed in several ways (fresh weight, dry weight, and per plant). Cotyledons, leaves, and stems were sampled separately. The sample, 50–100 gm. weighed accurately to one decigram, was cut in small pieces with a pair of scissors. Five-tenths of a gram of calcium carbonate (ppt. chalk) was sprinkled over it to neutralize the acidity of the solution, which would cause the inversion of sucrose and other changes; sufficient 95 per cent aldehyde free alcohol and water was added to bring the final concentration of alcohol to 80 per cent. The mixture was heated rapidly to boiling on the steam bath to stop enzyme action, and set

aside, carefully covered, for a day or longer. The liquid was then decanted off, filtered through a hardened filter, and the residue dried at 105° and pulverized. The powdered material was placed in a Vivian extractor and extracted with some of the alcoholic filtrate until the percolate was colorless. The volume of the total extract was made up to 1 liter with 80 per cent alcohol, and aliquots of this solution were used in the determination of the various soluble nitrogen and carbohydrate compounds, as follows:

### Chemical analyses of samples

SUBSTANCE DETERMINED

1. Insoluble residue
2. Solids in extract
3. Insoluble nitrogen
4. Soluble nitrogen
5. Nitrate nitrogen
6. Nitrites
7. Ammonia nitrogen
8. Amide nitrogen
9. Amino acid nitrogen
10. Reducing sugars
11. Sucrose
12. Starch

### METHODS

1. INSOLUBLE RESIDUE.—The material remaining from the final alcoholic extraction of the leaf tissue was dried at 100° C. and corrected for the added calcium carbonate.

2. SOLIDS IN EXTRACT.—Fifty cc. aliquots of the alcoholic extract were evaporated and the residue dried at 100° C.

3. INSOLUBLE NITROGEN.—The determination was made by the Kjeldahl process, on 1 gm. samples of the dried residue from the extraction. These were ground to pass through an 80 mesh sieve.

4. SOLUBLE NITROGEN OF EXTRACT.—Total nitrogen, including nitrates (preliminary reduction with Devarda's alloy), was determined, using 50 cc. aliquots of the alcoholic extract.

5. NITRATE NITROGEN.—This was determined by the phenol di-sulphonic acid colorimetric method as modified by BURRELL and PHILLIPS (1).

6. NITRITES.—Aliquots of the alcoholic extract were treated as in the nitrate determination, up to the point of being oxidized with sodium peroxide. At this stage they were neutralized with sodium hydroxide solution, and nitrites were determined colorimetrically, using the alpha naphthyl amine-sulphanilic acid reagent.

7, 8. AMMONIA AND AMIDE NITROGEN.—The Van Slyke Cullen urea apparatus was used, the ammonia being liberated by 52 per

cent potassium carbonate solution. Aeration was carried on for one hour into 0.02 N acid. Blanks were run simultaneously and the ammonia determined by titration. For amide nitrogen, the sample was subjected to a preliminary amide hydrolysis, and the total ammonia nitrogen was then found as previously described. Subtracting the ammonia nitrogen previously found present as such, gave the amide nitrogen.

9. AMINO ACID NITROGEN.—This was determined by the Van Slyke method, using the micro apparatus.

10. REDUCING SUGARS.—Picrate reduction and colorimetric estimation were used. The sample was prepared by the method recommended by THOMAS and DUTCHER (7). Analysis of the clear filtrate, however, was carried out by the method as described by WILLAMAN and DAVISON (8), making use also of their correction tables.

11. SUCROSE.—Part of the clarified filtrate from "reducing sugars" was treated by the standard Herzfeld procedure, and a determination of total sugars carried out by the method used for reducing sugars. Making use of the appropriate corrections in WILLAMAN and DAVISON'S paper, sucrose was calculated.

12. STARCH.—Two gram samples of the dried extracted residue were subjected to starch determinations according to the method of THOMAS (6).

## Discussion

MAGNESIUM DEFICIENCY.—Magnesium deficient plants were not noticeably different in appearance from the controls (table II). A very small supply of magnesium (largely derived from the cotyledons before they were removed) seemed sufficient for the usual development of the chlorophyll and growth of the plant for the 35-day periods used in these experiments. Some navy bean plants which were grown to the blossoming stage, however, did show a much decreased vigor and the leaves were lighter in color than those of the controls. There is not a great difference in chemical composition between the magnesium deficient and control plants. In general, the quantity of starch is slightly less in the magnesium deficient plants in both leaves and stems; soluble nitrogen is slightly greater in the leaves of the magnesium deficient plants and about the same in the stems; and insoluble nitrogen is consistently slightly less in both the

## TABLE II

ANALYSES OF NORMAL AND DEFICIENT SOY BEAN TISSUES; FRESH WEIGHT BASIS
MAGNESIUM DEFICIENCY

| Tissue | Age in days | No. of plants used | Fresh weight (gm.) | Percentage | | | | | | | | | | | | Nitrites |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Insoluble residue | Solids soluble 80 per cent alcohol | Dry matter | Insoluble nitrogen | Soluble nitrogen | Nitrate nitrogen | Ammonia nitrogen | Amide nitrogen | Amino acid nitrogen | Reducing sugars | Sucrose | Starch | |
| Cotyledons..... | 5 | 500 | 93.1 | 9.84 | 3.35 | 13.19 | 0.626 | 0.167 | ..... | 0.0016 | 0.0300 | 0.080 | 0.32 | 0.10 | 0.38 | + |
| Normal leaves.... | 35 | 200 | 106.9 | 11.92 | 3.76 | 15.68 | 0.553 | 0.141 | 0.021 | 0.0002 | 0.0082 | 0.031 | 0.24 | 0.36 | 0.34 | ++ |
| Deficient leaves.. | 35 | 200 | 100.55 | 11.18 | 3.24 | 14.42 | 0.470 | 0.163 | 0.024 | 0.0002 | 0.0052 | 0.042 | 0.21 | 0.30 | 0.29 | ++ |
| Normal stems.... | 35 | 200 | 60.9 | 10.59 | 1.80 | 12.39 | 0.189 | 0.097 | 0.039 | ..... | 0.0086 | 0.014 | 0.30 | 0.05 | 0.18 | + |
| Deficient stems... | 35 | 200 | 52.0 | 10.39 | 1.85 | 12.24 | 0.172 | 0.093 | 0.039 | ..... | 0.0101 | 0.010 | 0.22 | 0.04 | 0.13 | + |

## TABLE III

ANALYSES OF NORMAL AND DEFICIENT SOY BEAN TISSUES; FRESH WEIGHT BASIS
POTASSIUM DEFICIENCY

| Tissue | Age in days | No. of plants used | Fresh weight gm. | Percentage | | | | | | | | | | | | Nitrites |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Insoluble residue | Solids soluble 80 per cent alcohol | Dry matter | Insoluble nitrogen | Soluble nitrogen | Nitrate nitrogen | Ammonia nitrogen | Amide nitrogen | Amino acid nitrogen | Reducing sugars | Sucrose | Starch | |
| Cotyledons..... | 5 | 550 | 100.0 | 8.80 | 2.94 | 11.74 | 0.532 | 0.153 | ..... | 0.0018 | 0.032 | 0.071 | 0.28 | 0.15 | 0.32 | + |
| Normal leaves.... | 35 | 200 | 111.0 | 12.07 | 2.93 | 15.00 | 0.489 | 0.125 | 0.020 | 0.0002 | 0.005 | 0.045 | 0.24 | 0.28 | 0.30 | ++ |
| Deficient leaves.. | 35 | 200 | 100.6 | 12.22 | 3.46 | 15.68 | 0.442 | 0.147 | 0.023 | 0.0003 | 0.008 | 0.062 | 0.20 | 0.30 | 0.52 | ++ |
| Normal stems.... | 35 | 200 | 81.2 | 12.31 | 1.86 | 14.17 | 0.233 | 0.078 | 0.025 | ..... | 0.013 | 0.022 | 0.26 | 0.06 | 0.24 | + |
| Deficient stems... | 35 | 200 | 69.6 | 11.07 | 2.15 | 13.22 | 0.167 | 0.083 | 0.028 | ..... | 0.015 | 0.025 | 0.15 | 0.07 | 0.14 | + |

TABLE IV

ANALYSES OF NORMAL AND DEFICIENT SOY BEAN TISSUES; FRESH WEIGHT BASIS

CALCIUM DEFICIENCY

| TISSUE | AGE IN DAYS | NO. OF PLANTS USED | FRESH WEIGHT (GM.) | PERCENTAGE | | | | | | | | | | | | NITRITES |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Insoluble residue | Solids soluble 80 per cent alcohol | Dry matter | Insoluble nitrogen | Soluble nitrogen | Nitrate nitrogen | Ammonia nitrogen | Amide nitrogen | Amino acid nitrogen | Reducing sugars | Sucrose | Starch | |
| Cotyledons | 5 | 560 | 100.0 | 9.08 | 2.94 | 12.02 | 0.588 | 0.158 | 0.008 | 0.0022 | 0.040 | 0.083 | 0.35 | 0.12 | 0.21 | + |
| Normal leaves | 35 | 184 | 100.0 | 11.54 | 3.81 | 15.35 | 0.527 | 0.131 | 0.026 | 0.0008 | 0.007 | 0.046 | 0.28 | 0.33 | 0.31 | ++ |
| Deficient leaves | 35 | 252 | 100.0 | 11.83 | 4.28 | 16.11 | 0.344 | 0.108 | 0.049 | 0.0006 | 0.010 | 0.029 | 0.31 | 0.35 | 0.33 | ++ |
| Normal stems | 35 | 184 | 67.5 | 12.03 | 1.87 | 13.90 | 0.212 | 0.077 | 0.031 | 0.0004 | 0.011 | 0.019 | 0.21 | 0.08 | 0.27 | + |
| Deficient stems | 35 | 252 | 52.0 | 9.69 | 2.46 | 12.12 | 0.142 | 0.061 | 0.036 | 0.0005 | 0.006 | 0.013 | 0.23 | 0.13 | 0.15 | + |

stems and leaves of the magnesium deficient plants. There are many other slight variations in the quantities of the various substances determined, but these are quite apparent from the table. They are hardly sufficiently marked to justify any hypotheses to explain them until more data of this kind have been accumulated. Nodules were not developed on the roots of the soy beans in any of the experiments.

POTASSIUM DEFICIENCY.—Plants grown under a deficiency of potassium differed considerably in physical appearance from the controls (table III). The leaves and stems were a different shade of green, and many of the leaves were blotched with lighter shades. Over 50 per cent of the potassium deficient pumpkin plants died from a bacterial infection during the seedling stage; none of the control plants grown side by side with these were so affected. The most noticeable difference in chemical composition between potassium deficient and control plants is the accumulation of starch in the leaves of the former. In the stems, how-

ever, starch is less with the potassium deficient plants than with the controls. HARTWELL (3) has also observed a similar condition, while WIMMER (9) found a failure to produce sugar and starch with potassium deficient plants, but this may vary with the stage of development. The percentage of dry matter is slightly higher in the potassium deficient than in control leaves. Insoluble nitrogen is less in all cases in both the stems and leaves of the potassium deficient plants; soluble nitrogen is greater; and amino acid nitrogen is especially high as compared with the quantity present in control plants. Potassium seems to have some function in the translocation and utilization of starch, and to be of especial importance in the formation of proteins from carbohydrates and amino acids.

CALCIUM DEFICIENCY.—Calcium deficient plants differed more markedly from the controls than did the potassium or magnesium deficient plants (table IV). The calcium deficient plants were smaller, of a yellow color, and showed less root development than the controls. When the sand in the trays had dried slightly, the roots of the calcium deficient plants could be pulled up with ease, while the control plants could not. The roots of the calcium deficient plants were noticeably brownish in color, especially on drying, and many were losing their epidermis. ECKERSON noted a similar condition in plants grown in water cultures deficient in calcium, and explained it by assuming that insoluble calcium pectate was normally present, but that with the calcium deficient plants the calcium was replaced by magnesium and other ions to form soluble pectates. The most striking feature of the chemical composition is the accumulation of nitrate in the leaves of the calcium deficient plants. Insoluble nitrogen is considerably less and amino acid nitrogen is less in both the leaves and stems of the calcium deficient plants. Calcium may play some important rôle in nitrate reduction, although, as previously stated, caution must be observed against drawing too sweeping conclusions until more data can be accumulated.

## Conclusions

The use of deficient nutrient solutions for studying processes concerned in plant metabolism has been shown to have considerable possibilities. In these preliminary experiments the idea suggested at

the beginning of this paper was confirmed, namely, that certain elements play important rôles in different stages of synthesis occurring in plants. Also, when a certain essential element is markedly lacking, a process, as protein synthesis, may be greatly retarded at a certain stage, so that an abnormal accumulation of some intermediate product results. The products characteristic of the later stages of the synthesis are formed in abnormally small quantities.

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# AUTOBIOGRAPHY

I, Robin Charles Burrell, was born in Northfield, Ohio, March 14, 1896. I received practically all of my secondary school education in the public schools of Alliance, Ohio; my undergraduate education at Mount Union College, Alliance, Ohio, from which I obtained the degree of Bachelor of Science in 1918. The years 1919-1921 were spent at Ohio State University, from which institution I received the degree of Master of Arts. During this time I was an Assistant in Chemistry under Dr. Wm. Lloyd Evans. From 1921 to 1923 I was Associate Professor of Chemistry at the University of Richmond, Richmond, Virginia. I then returned to Ohio State University where, after a two years' residence on a University Instructorship, I received the degree of Doctor of Philosophy at the end of the Summer Quarter of 1925.